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Metabolic Syndrome Impairs Notch Signaling and Promotes Apoptosis in Chronically Ischemic Myocardium

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Abstract

Objective—Impaired angiogenesis is a known consequence of metabolic syndrome (MetS), however, the mechanism is not fully understood. Recent studies have shown that the Notch signaling pathway is an integral component of cardiac angiogenesis. We tested in a clinically relevant swine model the effects of MetS on Notch and apoptosis signaling in chronically ischemic myocardium.

Methods—Ossabaw swine were fed either a regular diet (CTL, n=8) or a high-cholesterol diet (MetS, n=8) to induce MetS. An ameroid constrictor was placed to induce chronic myocardial ischemia. Eleven weeks later, animals underwent cardiac harvest of the ischemic myocardium.

Results—There was down-regulation of pro-angiogenesis proteins Notch2, Notch4, Jagged2, Ang1 and ENOS in the MetS group compared to CTL. There was also up-regulation of pro-apoptosis protein Caspase8, and down-regulation of anti-angiogenesis protein pFOX03, and pro-survival proteins pP38 and HSP90 in the MetS group. Cell death was increased in the MetS group compared to CTL. Both CTL and MetS groups had similar arteriolar count and capillary density, and Notch3 and Jagged1 were both similarly concentrated in the smooth muscle wall in both groups.

Conclusions—MetS in chronic myocardial ischemia significantly impairs Notch signaling by down regulating Notch receptors, ligands and pro-angiogenesis proteins. MetS also increases apoptosis signaling, decreases survival signaling and increases cell death in chronically ischemic myocardium. Although short-term angiogenesis appears unaffected in this model of early MetS,

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the molecular signals for angiogenesis are impaired, thus suggesting that inhibition of Notch signaling may underlie decreased angiogenesis in later stages of MetS.

Keywords

coronary disease; ischemia; molecular biology

Introduction

Metabolic syndrome is a cluster of metabolic derangements including obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension. Metabolic syndrome substantially increases the risk of cardiovascular disease and mortality.[1, 2] Despite advancements in surgical technique and percutaneous interventions, patients with hypertension, hyperlipidemia and diabetes have a significantly higher mortality after angioplasty, coronary stenting and coronary artery bypass grafting.[3] Promising results from animal studies in therapeutic revascularization with growth factor, gene or cell therapy has been met with disappointing results in humans with minimal clinical improvements in myocardial angiogenesis.[4] Not surprisingly, young healthy animals with chronic myocardial ischemia are better able to adapt to ischemic insult, whereas older patients with multiple comorbidities, including MetS, have limited myocardial adaptability to ischemia. The discordant results between positive animal studies and negative clinical results highlights the need to understand the effects of MetS on molecular angiogenic signaling pathways using a clinically relevant animal model.

Ossabaw miniswine are a breed of feral pigs that were isolated on Ossabaw Island off the coast of Georgia almost 500 years ago by Spanish explorers. This pig breed has been identified as an excellent model for metabolic syndrome due to its “thrifty genotype”, which allowed these pigs to adapt to the harsh island conditions by storing large amounts of fat during the feasting period.[5] When sedentary and fed a high-fat, high-calorie, atherogenic diet, these animals develop profound obesity and all the hallmark features of MetS and are a useful model to sudy MetS and coronary artery disease.[5-7]

We recently demonstrated that animals with chronic myocardial ischemia and perivascular VEGF had significantly improved neovascularization and Notch receptor and ligand expression.[8] The Notch signaling pathway is an evolutionarily conserved pathway that is important for many processes including cell fate determination differentiation, proliferation, apoptosis, and regeneration.[9] Studies have shown that postnatal Notch signaling is critical for angiogenesis.[10, 11] There is growing interest in the clinical utility of Notch modulators to suppress angiogenesis in tumors, and promote angiogenesis in ischemic myocardium.[12] While research in the role of Notch signaling in developmental biology is longstanding, the role of Notch in mature myocardium in response to ischemia and metabolic syndrome is largely unknown. The purpose of this study is to examine the effects of metabolic syndrome on Notch signaling in response to chronic myocardial ischemia in the clinically relevant Ossabaw model of early MetS.

Materials and Methods

ANIMAL MODEL

Sixteen intact male Ossabaw miniswine (Purdue Ossabaw Facility, Indiana University, Indianapolis, IN) were split into two groups according to diet at 6 weeks of age. The control group was fed 500g/day of regular chow (CTL, n=8). The high-cholesterol animals were fed 500g/day of high-cholesterol chow consisting of (by weight) 4% cholesterol, 17.2% coconut oil, 2.3% corn oil, 1.5% sodium cholate, and 75% regular chow (Sinclair Research, Columbia, MO) (MetS, n=8). After 9 weeks of diet initiation, all animals underwent surgical placement of an ameroid constrictor to induce chronic myocardial ischemia (see surgical interventions). Postoperatively, all animals continued on their respective diets. Eleven weeks after ameroid constrictor placement, all animals underwent euthanasia and cardiac tissue harvest. All animals were observed to ensure complete consumption of food, had unlimited access to water, and were housed in a warm non-stressful environment for the duration of the experiment.

SURGICAL INTERVENTIONS

Anesthesia—Anesthesia was induced with an intramuscular injection of telazol (4.4 mg/kg). Animals were endotracheally intubated, mechanically ventilated at 12 - 20 breaths per minute, and general anesthesia was maintained with a gas mixture of oxygen at 1.5 - 2 liters/min and 0.75-3.0% isoflurane.

Ameroid Constrictor Placement—Animals were given a single dose of intravenous enrofloxacin (5 mg/kg) for antibiotic prophylaxis and general anesthesia was induced and maintained. Animals were prepped and draped in the usual sterile fashion. The heart was exposed through a left mini-thoracotomy through the fourth intercostal space and pericardiotomy. The left atrial appendage was retracted and the left circumflex artery was dissected at the take off of the left main coronary artery. A titanium ameroid constrictor (1.75-2.25 mm internal diameter) ameroid constrictor was placed around the proximal left circumflex artery, just after its take off from the left main coronary artery. (Research Instruments SW, Escondito, CA). The pericardium was loosely re-approximated with interrupted 4-0 neurolon sutures (Ethicon, Somerville, NJ) followed by a layered closure of the surgical incision. Post-operative pain was controlled with a single dose of intramuscular buprenorphine (0.03 mg/kg) and 72 hour fentanyl patch (4 g/kg). All animals received 325 mg of aspirin daily starting 1 day pre-operatively and continuing for a total of 5 days for prophylaxis against thrombo-embolic events. All animals continued perioperative antibiotics: enrofloxacin 68 mg orally daily for 5 days.

Cardiac Harvest—Under general anesthesia, the heart was exposed via a median sternotomy and animals were euthanized by exsanguinations. Cardiac tissue from the ischemic territory in the left circumflex artery distribution was collected for further analysis. The Institutional Animal Care and Use Committee of the Rhode Island Hospital approved all experiments. Animals were cared for in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 5377-3 1996).

IMMUNOHISTOCHEMICAL STAINING FOR ANGIOGENESIS

Frozen myocardium was sectioned (10-m-thickness) and fixed in 10% formalin for 10 minutes. Sections were blocked with 1% bovine serum albumin in phosphate buffered saline for 1 hour at room temperature and incubated with antibodies against porcine endothelial marker CD-31 (R&D Systems, Minneapolis, MN) and smooth muscle actin (Sigma Aldrich, St. Louis, MO), followed by the appropriate alexa-fluor conjugated antibody (Jackson ImmunoResearch, West Grove, PA) for 45 minutes. Slides were then mounted with DAPI-containing medium Vectashield (Vector Laboratories, Burlingame, CA). Images were captured at X20 magnification with a Nikon E800 Eclipse microscope (Nikon, Tokyo, Japan) at the same exposure in three random fields. Capillaries were defined as structures between 5-25 m^2 in cross-sectional area and arterioles were defined by co-localization of smooth muscle actin (red) and CD-31 (green) staining. Arteriolar density and capillary density was measured using Image J software (National Institutes of Health, Bethesda, MD). The percent capillary and arteriolar density for each animal was averaged from the three randomly selected myocardial tissue sections.

PROTEIN EXPRESSION

Forty micrograms of the Radio-Immunoprecipitation Assay (Boston BioProducts, Ashland, MA) soluble fraction of myocardial lysates were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis 3-8% Tris-acetate gel (NuPage Novex Mini Gel, Invitrogen, Carlsbad, CA) for molecular weight targets >100 kilodaltons and 4-12% Bis-Tris gels for molecular weight targets <100 kilodaltons (NuPage Novex Mini Gel, Invitrogen). The protein was then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA) and incubated overnight at 4 °C with primary antibodies at dilutions recommended by the manufacturer against Notch1, Notch2, Notch3, Notch4, Jagged1, Jagged2, Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Vascular Endothelial Cadherin (VECadherin), Endothelial Nitric Oxide Synthase (ENOS), Transforming Growth Factor β (TGFβ), Caspase8, Caspase3, Cleaved Caspase3, Forkhead Box Transcription Factor 03 (Fox03), phosphorylated Fox03, Apoptosis Inducing Factor (AIF), P38, phosphorylated P38 (pP38), Heat Shock Protein 90 (HSP90) (all from Cell Signaling, Danvers, MA) and VEGFR3 and angiopoietin 1 (Ang1) (both from Abcam, Cambridge, MA). Membranes were incubated with the appropriate horseradish peroxidase-linked secondary antibody for one hour at room temperature (Jackson ImmunoResearch, West Grove, PA). Immune complexes were visualized with enhanced chemiluminescense and images were captured with a digital camera system (G-Box, Syngene, Cambridge, England). Band densitometry was quantified as arbitrary light units using Image-J software. All membranes were probed with GAPDH (Cell Signaling) to correct for loading error.

IMMUNOHISTOCHEMICAL STAINING FOR NOTCH SIGNALING PROTEINS

Frozen myocardium was sectioned (10-m-thickness) and fixed in 10% formalin for 10 minutes. Sections were blocked with 1% bovine serum albumin in phosphate buffered saline for 1 hour at room temperature and incubated with antibodies against Notch2, Notch3, Notch4 or Jagged1 (Cell Signaling) and smooth muscle actin (SMA, Sigma Aldrich) co-staining, followed by the appropriate alexa-fluor conjugated antibody (Jackson

ImmunoResearch) for 45 minutes. Slides were then mounted with Vectashield. Images were captured at X20 magnification with a Nikon E800 Eclipse microscope at the same exposure in three random fields. Immunofluorescence mean intensity was measured using Image J software. The immunofluorescence mean intensity for each animal was averaged from the three randomly selected myocardial tissue sections.

TUNEL STAINING

Frozen myocardium from the ischemic territory was sectioned (10-m-thickness). Apoptotic cells were identified using the commercially available ApopTag detection kit (Millipore, Billerica, MA) according to the manufacturer's instructions. Images were captured at X20 magnification using Aperio ScanScope technology (Vista, CA) in three random fields from each animal. The percentage of TUNEL-positive cardiomyocytes per high powered field was measured using Image J software. The percent cell death per high powered field for each animal was averaged from the three randomly selected myocardial tissue sections.

DATA ANALYSIS

All results are reported as mean ± standard error of the mean. A Student's T-test was used to compare the means with GraphPad Prism 5.0 Software (GraphPad Software Inc., San Diego, CA). Differences with a p value <0.05 were considered statistically significant. Protein expression is reported as fold change compared to CTL.

Results

Animal Model

All animals included in the analysis survived the entire experiment. One animal in the CTL group died on post-operative day 8, and 1 animal in the MetS group died on post-operative day 2. Necropsy was unremarkable, and it was assumed that both animals died of an acute ventricular arrhythmia. The animals that did not survive to completion of the experiment were excluded from analysis and replaced with new animals. We previously reported that there was no difference in myocardial perfusion or contractility.[13] We also reported that the animals in the MetS group developed key components of metabolic syndrome including obesity (BMI 31.9 vs 35.1 in CTL vs MetS $p<0.01$), hypertension (MAP 85 mmHg vs 112 mmHg in CTL and MetS respectively $p=0.02$), dyslipidemia (67.9 mg/dL vs 343 mg/dL total cholesterol in CTL and MetS respectively $p<0.01$) and glucose intolerance in response to dextrose infusion (44, 149, 82 mg/dL blood glucose in CTL group vs 60, 214, 130 mg/dL in the MetS group at 0 min, 30 min, 60 min, respectively, after dextrose infusion; $p<0.01$).[13]

Vessel Density in Chronically Ischemic Myocardium

There was no difference in capillary density in the chronically ischemic myocardium as measured by immunohistochemical staining for CD31 (9.53±0.72 and 10.78±0.74 percent area/high power field CTL and MetS respectively $p=0.28$). Also, there was no difference in arteriolar count as measured by immunohistochemical co-staining of CD31 and smooth muscle actin (22.91±1.18 and 23.73±0.93 count/high power field CTL and MetS respectively $p=0.59$) (Figure 1).

Angiogenesis Protein Expression in Chronically Ischemic Myocardium

We analyzed the myocardial lysates of the chronically ischemic myocardium for expression of all 4 mammalian Notch receptors (1-3) and 2 Notch ligands (Jagged 1 and 2). In the METS group, there was a decrease in expression of Notch2, Notch4, and Jagged2 compared to CTL. There was also decreased expression of pro-angiogenesis proteins Ang1 and ENOS in the MetS group compared to CTL. There was no difference in the expression of Notch1, Notch3, Jagged 1, VEGFR1, VEGFR2, and TGFβ in the CTL and MetS groups (Table 1).

Notch Receptor and Ligand Staining in Chronically Ischemic Myocardium

We performed immunohistochemical staining of transmural sections of the ischemic myocardium to determine the histologic location of the Notch2, Notch3 and Notch4 receptors by co-staining with smooth muscle actin. Smooth muscle actin stains vascular smooth muscle, which is located in the wall of arteries and arterioles. Notch receptors Notch2, Notch3, and Notch4, and Notch ligand Jagged1 (green) were highly concentrated in the smooth muscle wall and co-localize with smooth muscle actin (red) (Figure 2 and Figure 3). Although there was no difference in overall Notch3 intensity in the CTL and MetS groups, there was a significant decrease in Notch2 and Notch4 intensity in the MetS group compared to CTL. There was a trend toward decreased Jagged1 immunoflurecence in the MetS group compared to CTL, but it did not reach statistical significance.

Apoptosis and Survival Protein Expression in Chronically Ischemic Myocardium

We analyzed myocardial lysates of the chronically ischemic myocardium for the expression of apoptosis and survival signaling proteins. We found that in the MetS group, there was up-regulation of pro-apoptosis protein Caspase8 and down-regulation of the inhibited form of pro-apoptosis protein Fox03, pFox03 compared to CTL. There was also significant down-regulation of pro-survival proteins pP38 and HSP90 in the MetS group compared to CTL. There was also increased expression of pro-apoptosis proteins Cleaved Caspase3 and AIF in the MetS group but it did not reach statistical significance (p=0.06 and 0.09 respectively) compared to CTL (Table 2). There was no difference in the expression of pro-apoptosis proteins Caspase3, Fox03 or pro-survival protein P38.

TUNEL Staining in Chronically Ischemic Myocardium

We sampled the chronically ischemic myocardium and performed TUNEL staining to quantify cell death in the CTL and MetS group. We found that there was a significant increase in cardiomyocyte cell death in the MetS group compared to CTL (2.73±0.36 vs 1.71±0.34 p=0.05 respectively) (Figure 4).

Discussion

Coronary collateralization in response to chronic myocardial ischemia is an important endogenous mechanism to preserve myocardial function, limit infarct size, and improve long term survival. It is therefore critical to understand the molecular basis for how MetS inhibits coronary collateralization and angiogenesis. In this clinically relevant swine model of chronic myocardial ischemia, we found that early MetS attenuated Notch, angiogenesis, and survival signaling, and up-regulated apoptosis signaling in chronically ischemic

myocardium. Previously, we demonstrated that feeding Ossabaw swine a high-fat, high-calorie, atherogenic diet resulted in all of the hallmark components of MetS including obesity, insulin resistance, glucose intolerance, elevated blood pressure and hyperlipidemia.[13, 14]

The Notch signaling pathway is a short-range communication system between two physically adjacent cells, so called juxtacrine signaling. In mammals, there are 4 Notch receptors: Notch 1-4, and 5 receptors: Delta Like (DLL)1,3,4 and Jagged 1 and 2. The vascular endothelium only expresses receptors: Notch1, Notch2, Notch4, and ligands Jagged1, Jagged 2, DLL1 and DLL4.[12] Notch3 is predominantly expressed in the adult arterial smooth muscle wall.[15] Notch signaling is an integral part of both angiogenesis and vasculogenesis, and is required for arterial and venous blood vessel differentiation.[16] The Notch pathway also maintains endothelial homeostasis by 1) modulating new blood vessel sprouting via DLL4/Notch1, 2) Protecting endothelial cells from TNFα mediated apoptosis via Notch4, and 3) regulating bone marrow endothelial progenitor cell proliferation, apoptosis and migration to ischemic tissue via Notch1.[12]

The Notch pathway has been proposed as a possible target for therapeutic angiogenesis and cardioprotection, however studies in a large animal model with clinically relevant comorbidities, such as MetS, are lacking.[12] In the present study, we demonstrate that MetS has an inhibitory effect on Notch signaling. We found that Notch receptors Notch2, Notch3, and Notch4, and Notch ligand Jagged1 were primarily concentrated in the vascular smooth muscle wall, and co-localized with smooth muscle actin. This was an expected result given that Notch3 is expressed in vascular smooth muscle cells. There was also decreased Notch2 and Notch2 staining, and a trend towards decreased Jagged1 tissue staining in the MetS group compared to the control, suggesting that there is decreased Notch signaling in the vascular smooth muscle wall in animals with MetS. Jagged1 has been identified as an important Notch target during arterial wall assembly with Jagged1 inhibition resulting in arterial defects and impaired vascular smooth muscle differentiation.[17] Our results suggest that perhaps MetS inhibits Notch signaling angiogenic response to chronic myocardial ischemia. We also reported that there was down-regulation of pro-angiogenic proteins eNOS and Ang1. Previous studies have demonstrated a direct link between Notch signaling and eNOS activity.[18, 19] Notch activation activates eNOS, which induces NO synthesis. In an animal model of hindlimb ischemia, Notch inhibition in endothelial cells reduced reperfusion and NO generation.[18] Ang1 is an angiogenic factor that also activates the pro-survival signaling pathway and initiates stem cell homing in the setting of myocardial ischemia.[20] In a porcine model of myocardial infarction, co-expression of VEGF and Ang1 was shown to increase angiogenesis, myocardial perfusion, and cardiomyocyte proliferation in ischemic myocardium.[21] In the current study, MetS had an inhibitory effect on the normal angiogeneic response to ischemia by down-regulating eNOS and Ang1.

It is somewhat surprising that in this model of early MetS in Ossabaw swine we did not observe a reduction in collateral dependent myocardial perfusion, as we have reported using other strains of pigs.[22, 23] This may be specific to the Ossabaw pigs, and in part as a result of increased vascular relaxation observed using these pigs in early stage MetS.[13] Perhaps the increased microvascular relaxation may be due to up-regulation of the PPAR pathway, and it

may also be related to the “thrifty genotype”, which enabled this pig breed to survive in the feast/famine ecology on Ossabaw Island despite their predilection to obesity and coronary artery disease. It may also be the case that this transient improvement in microvessel reactivity that we previously reported also occurs in humans in early metabolic syndrome. Later stage MetS in Ossasbaw swine, in contrast, is associated with decreased endothelium-dependent relaxation and coronary flow reserve, thus indicating the progression of MetS severity.[6, 24-26] Given the decreased expression of Notch and angiogenesis signaling proteins, one would expect that there would also be decreased vessel density. In this study however, we did not find any difference in arteriolar or capillary density in the MetS group compared to CTL. This finding may be due to the fact at these animals were supplemented with a high fat diet for a relatively short period of time and developed early-stage MetS. Importantly, molecular changes in angiogenesis protein expression precede histologic changes and reductions in vessel count. If the animals were fed the high-fat diet for a longer period of time, and had long-standing chronic MetS, the molecular down-regulation of Notch and angiogenesis signaling would manifest in a histologically visible reduction in angiogenesis. Indeed, Trask et al. reported decreased capillary density in Ossabaw pigs that were older and had MetS for a longer duration.[24]

In addition to its pro-angiogenic properties, Notch signaling has also been found to modulate apoptosis and survival signaling. In cell culture, pro-apoptotic TNFα administration down-regulated Notch4 activity.[27] Notch4 also increases expression of Bcl2, thereby protecting endothelial cells from lipopolysaccharide-triggered apoptosis.[28] Notch1 signaling in rat cardiomyocytes reduces apoptosis in ischemic preconditioning by up-regulating Bcl2 and down-regulating Bax.[29] Thus, Notch signaling could be used to promote endothelial generation and angiogenesis, and inhibit endothelial and cardiomyocyte apoptosis. Studies in oncologic biology have shown that Notch signaling inhibits apoptosis through the AKT pathway in neoplastic tissues.[30, 31] In this study, we examined the effect of metabolic syndrome on apoptosis-survival signaling in ischemic myocardium. There was a significant decrease in pro-survival proteins pP38 and HSP90, a decrease in the inhibited form of pro-apoptosis protein pFox03, and up-regulation of pro-apoptosis protein Caspase 8 in the MetS group. There was also a trend towards increased expression of pro-apoptosis proteins AIF and cleaved Caspase3 in the MetS group. The increase in pro-apoptosis protein expression and decrease in expression of survival proteins in the MetS group was supported by TUNEL staining, which demonstrated that there was increased cell death in the MetS group compared to CTL. These results suggest that metabolic syndrome promotes apoptosis signaling and increases cell death in the setting of chronic myocardial ischemia. Although we previously reported that there was no difference in cardiac function, perhaps long-term MetS-mediated cell death may result in decreased contractility and reduced adaptability to myocardial ischemia.[8] Our finding of decreased Notch signaling in this early stage MetS is consistent with these molecular signals being causally related to decreased angiogenesis in later stage MetS.[24]

Metabolic syndrome poses a significant public health dilemma and its prevalence is increasing with no signs of slowing.[32] Notch signaling has been established as an integral component of angiogenesis and cell survival in the postnatal heart. Elucidating this complex signaling network may assist in identifying possible Notch targets and its applicability and

efficacy in human pathology. We provide evidence that MetS inhibits Notch signaling and promotes cell death in chronically ischemic myocardium, which may be one way that MetS negatively impacts overall cardiac health and increases risk for developing cardiovascular disease.

Study Limitations

There are several limitations in the presentation and analysis of this study. Although the animals in this study had early MetS, the known vascular consequences of MetS were not demonstrated in the chronically ischemic myocardium, namely there was no difference in vessel count or in microvessel relaxation. In future studies, it would be helpful to repeat the study with multiple harvest points to establish a time course for the molecular and histologic changes in angiogenesis in the setting of ischemia and early and established MetS. Furthermore, although we found differences in Notch expression in this animal model of early metabolic syndrome and chronic ischemia, causality with angiogenesis has not been clearly established. Future studies with directed up-regulation of Notch receptor or ligands are warranted to further elucidate the relationship between Notch signaling, metabolic syndrome and chronic myocardial ischemia.

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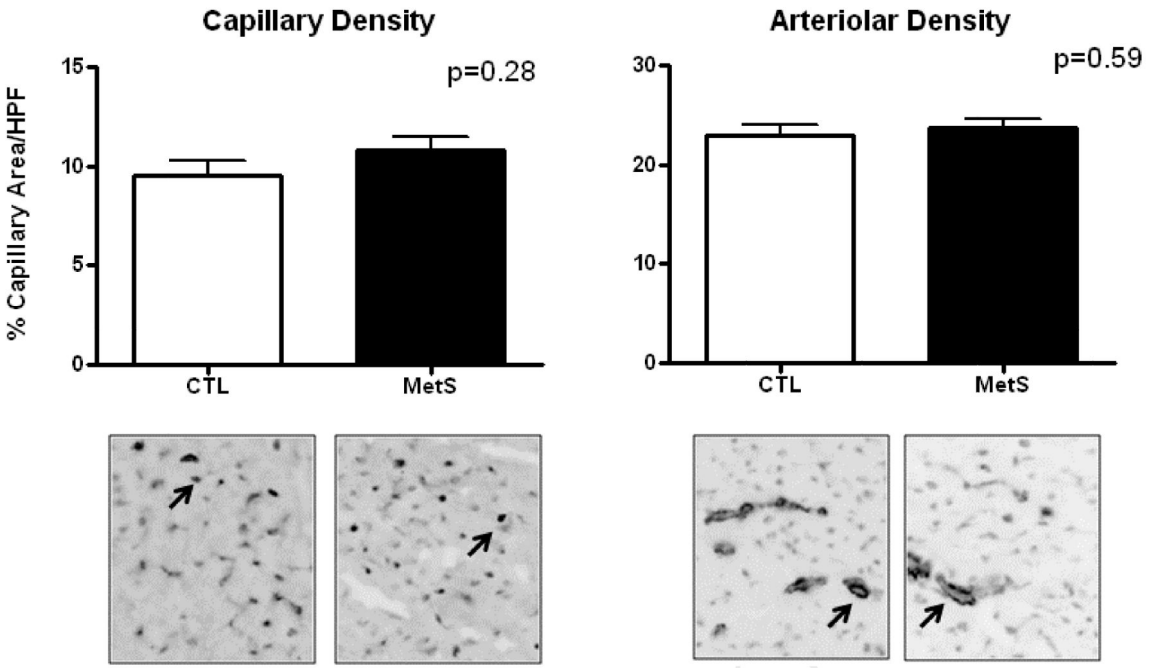

Figure 1.

Vessel Density in Chronically Ischemic Myocardium. Arrows in left panel indicate capillaries. Arrows in right panel indicate arterioles. CTL: Ossabaw Control. MetS: Ossabaw atherogenic diet.

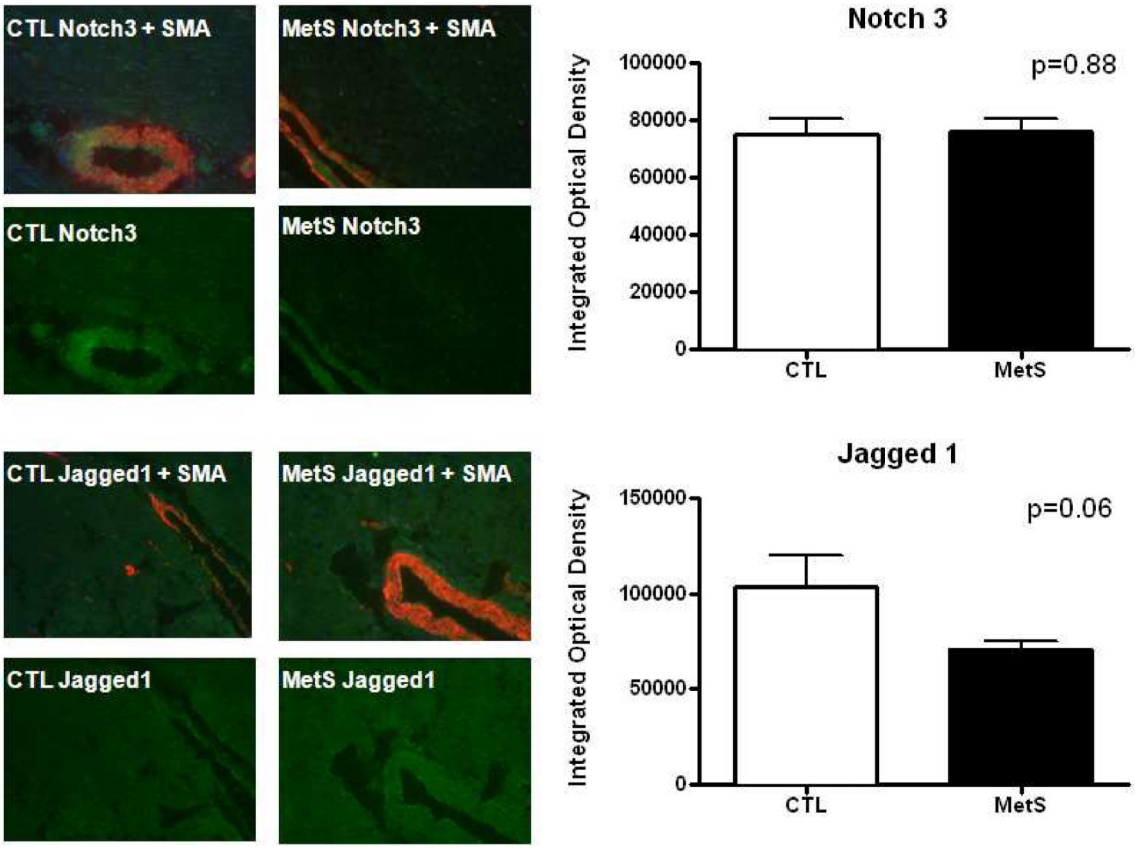

Figure 2.

Notch Receptor and Ligand Staining in Chronically Ischemic Myocardium. Notch receptor 3 (Notch3) in green in the top panel. Smooth muscle actin (SMA) in red. Notch ligand Jagged1 in green in the bottom panel. CTL: Ossabaw Control. MetS: Ossabaw atherogenic diet.

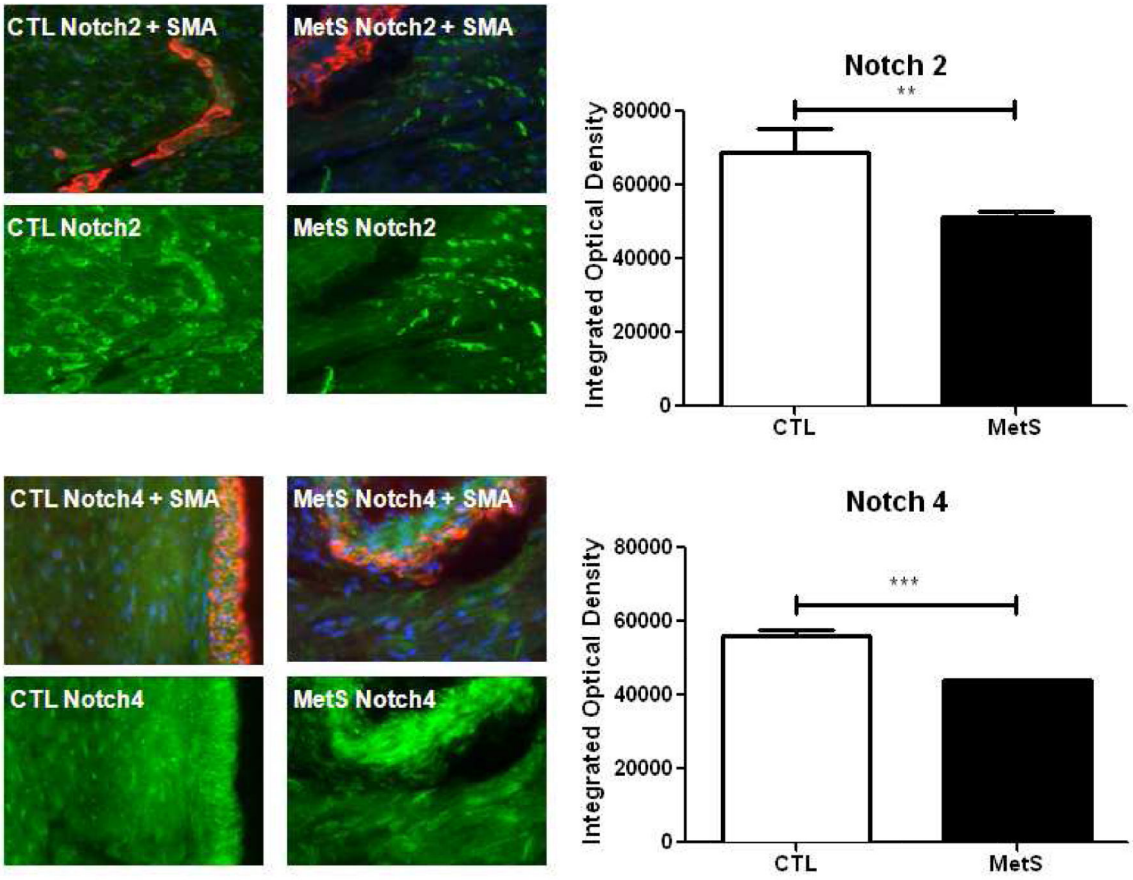

Figure 3.

Notch Receptor Staining in Chronically Ischemic Myocardium. Notch receptor 2 (Notch2) in green in the top panel and Notch receptor 4 (Notch4) in green in the bottom panel. Smooth muscle actin (SMA) in red. CTL: Ossabaw Control. MetS: Ossabaw atherogenic diet.

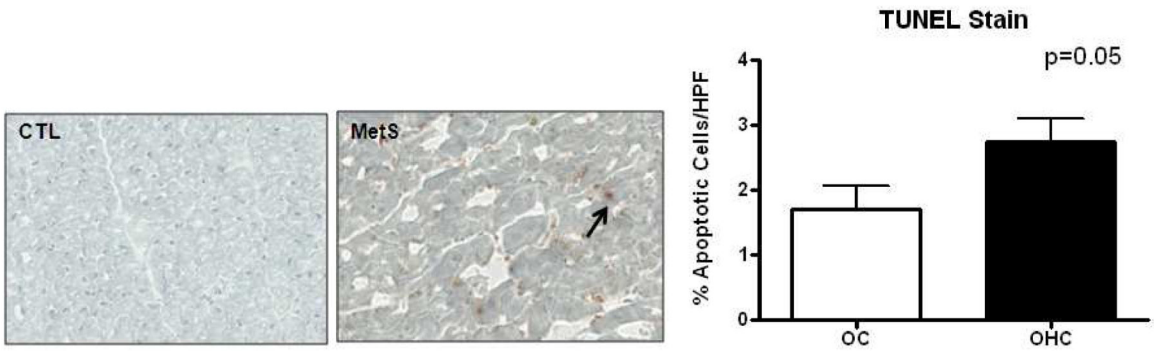

Figure 4.

TUNEL Staining in Chronically Ischemic Myocardium. Brown spots (arrow) in nuclei indicates cell death. CTL: Control. MetS: Ossabaw atherogenic diet.

Table 1
Angiogenesis Protein Expression in Chronically Ischemic Myocardium

	CTL	MetS	p Value
Notch1	1.0±0.132	0.81±0.053	0.20
Notch2	1.0±0.105	0.64±0.096	0.03
Notch3	1.0±0.076	1.11±0.088	0.38
Notch4	1.0±0.128	0.66±0.050	0.02
Jagged1	1.0±0.143	1.17±0.151	0.43
Jagged2	1.0±0.050	0.79±0.083	0.05
VEGFR2	1.0±0.082	1.12±0.127	0.43
VEGFR3	1.0±0.100	1.05±0.107	0.76
VE Cadherin	1.0±0.052	0.82±0.113	0.18
Ang1	1.0±0.070	0.72±0.045	0.01
ENOS	1.0±0.038	0.51±0.126	0.003
TGFβ	1.0±0.037	0.98±0.070	0.77

CTL: Control. MetS: Ossabaw atherogenic diet.

Values reported as fold change ± standard error of the mean compared to CTL.

Table 2
Apoptosis and Survival Protein Expression in Chronically Ischemic Myocardium

	CTL	MetS	p Value
Caspase8	1.0±0.051	1.19±0.051	0.02
Caspase3	1.0±0.065	1.04±0.093	0.72
Cleaved Caspase3	1.0±0.162	1.55±0.210	0.06
Fox03	1.0±0.025	0.95±0.033	0.27
pFox03 (Ser253)	1.0±0.039	0.84±0.040	0.01
AIF	1.0±0.108	1.32±0.136	0.09
P38	1.0±0.028	1.03±0.053	0.61
pP38 (Thr180/Tyr182)	1.0±0.052	0.80±0.029	0.01
HSP90	1.0±0.038	0.85±0.052	0.04

CTL: Control. MetS: Ossabaw atherogenic diet.

Values reported as fold change ± standard error of the mean compared to CTL.